Histogegesis of the Female Germ Cell in Orthoptera with Special Reference to Mermiria Bivitata

by Edwin C. Schmitt

May 15, 1913

Submitted to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Degree of Master of Arts

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INTRODUCTION:-

Comparatively little work has been done on the female germ cells of Orthoptera for quite a number of years, although the life cycle of any one species has not yet been completed. Textbooks on Entomology fail to give an account of the complete life history of the same. True, they discuss the ovary, method of deposition of the egg by the female and the later stages in embryology. The earlier stages are omitted and conditions there can only be surmised. Maturation and fertilization have,as yet, had no description and have to my knowlege never been found.

This deficiency, however, is due not to a lack of an investigative spirit among scientists, but rather to the difficulty in preparing the material in a manner suitable for microscopic study. The eggs are very difficult to section for as soon as the egg cell begins to deposit yolk material in its cytoplasm it is of little value for microscopic observation, for, in sectioning, the yolk granules sprinkle out and hence destroy any evidence as to the presence of germ cells. However, when the embryo is partly developed it can be dissected away from the yolk and then be prepared and studied separately. The yolk also undergoes a change at this time, it being transformed to protoplasm by a group of cells called vitellophags, having this specific function.

The purpose of this work is to discover,,if possible,

a suitable technique for fixing the Orthoptera eggs in such a way that they can be sectioned without loss of yolk. This being done to see, if possible, the condition of the nucleus in the growing egg; working out the maturation and fertilization divisions and the early embryology. I will first review some literature on Insect Embryology before describing the technique and observation.

REVIEW OF LITERATURE:- A number of papers have been devoted to the development of insect germ cells and their early embryology. A few have been quite successful in working out the phases of their development, as Wheeler, ('89) in his work on Blatta; Hegner, ('06) in his work on Chrysomelid eggs. Wheeler in ('92) again came forth with a contribution to Insect Embryology. This time on Xiphidium ensiferum, belonging to the order of Orthoptera. I deem it advisable to give the following brief description of the above mentioned contributions, in so far that it can be used for comparing descriptions appearing on the latter pages of this paper.

Wheeler did not study the preblastodermic stages, being unable to obtain sections suitable for microscopic observations. He merely suggests that they probably resemble the corresponding stages in Blatta. The blastoderm, when formed, consists of a thin sheet of cells

that have in part reached the surface of the yolk from the inside of the egg and are in part derived by tangential division. He was not able to determine whether the future vitellophags, which he found scattered through the yolk, were the result of division in the cells of the blastoderm or whether they were cells inhibited in their travel to the periphery of the yolk.

The blastodermic cells are polygonal in shape, much flattened and uniform in size and distribution and from which come the first evidences of the embryo: "Those (blastodermic cells) on the center of the ventral surface of the egg soon begin to change their dimensions; from being broad and flat, they become more nearly cubical, their lenticular nuclei again assuming a spherical shape, which they had in preblastodermic stages. These changes take place over a limited and somewhat oval area and result in the formation of the ventral plate".

This ventral plate has a few centers where the cells are more or less closely aggregated. Wheeler distinguishes four such centers, two for the cephalic lobes, one for the caudal end and one for the indusium. The latter, he thinks, is formed separately and joins the body of the embryo at a slightly later period, when the other centers have become united.

The procephalic lobes are succeeded by the strap-shaped body. This occurs when the centers above mentioned have united by tangential division and the ventral plate has become elongated by the same process. Body segments next make their appearance and are formed from before backwards. Through the elongation of the embryo the cephalic end of the same reaches the indusium and unites with it.

Wheeler then describes at some length the later development of the embryo, but since that would be of litle value in this paper I will not review that part of it here for this work is primarily concerned with the earlier stages.

Hegner('99) observed a polar disc composed of dark staining granules at the broad pole of Chrysomelid eggs. These,he said, were incorporated in the pole cells which, with the development of the embryo find their way unchanged into the abdominal cavity, where they arrange themselves into two lateral groupes in the dorsal region of the embryo, later to give rise directly to the genital anlage. Whence these pole cells have their origin he does not explain.

Henneguy quotes several works in which the intra-vitelline cells were observed: "Les auteurs qui apres

Bobretsky et Weismann, ont étudié la segmentation de l'oeuf des insects, Korotneff (1885), chez Gryllotalpa; Ayers (1884), chez Oecanthus; Grassi (1884), chez Apis; Patten (1884), chez les Pyryganides, ont constaté l'existence de noyaux intravitelluis se rendant à la périphérie de l'oeuf, mais toujours sans déterminer l'origne de ces noyaux. Henking (1886) soutenait encore la disparition complète de la vésicule germinative et la formation libre des noyaux de segmentation, résulte de l'union du noyau mâle et du noyau femelle, comme chez les autres animaux, Heider (1889), chez l'Hydrophile; Voeltzkow (1889), chez la Mouche; Wheeler (1889), chez la Blatta, ont confirmé le avancé per Blochmann. In as much as these investigators found vitelline cells and traced them to the germ cell, the material they worked with contained a compaaratively small amount of yolk, hence the difficulty connected with their work was reduced.to a consiserable degree.

MATERIAL AND TECHNIQUE:- The eggs for this work were collected during the summer of 1912. Yhey were obtained from one individual, which was found in the fields with its ovipositor in the ground ready for depositing eggs, but had as yet deposited none. It was then put into captivity and the next morning eighteen eggs were found

deposited. They were quite elongated and rod shaped, having a length of five mm. and a diameter of one and five tenths mm. A concave dorsal and a convex ventral side were easily recognizable. One pole possessed a very noticable cap like structure, the micropyle. This is the broad pole and lies toward the caudal end of the animal. The capsule, composed of a leathery chitinous substance, was of a brownish color, appeared rough and felt somewhat raspy to the touch.

These mature eggs were kept in as near a natural environment as possible by putting them in earth and keeping them in sunshine. Every day, beginning with the day they were found, two eggs were taken from the lot and tipped. i.e. with a pair of sharp scissors just enough of the capsule was removed from one pole of the egg to expose the yolk, which possesses such a consistency that it will not flow out of the capsule of its own accord. It was then immediately dropped into Bouin's fluid and fixed in toto. I believe this tipping offers an advantage for it gives the fixing agent an opportunity to act immediately and directly upon the yolk and rapid fixation is desired.

Some eggs were taken from the abdominal cavity of grasshopper nearly mature. The method for fixing these was the same. After remaining in Bouin's for several days the eggs were run thru 30%, 50% and left in 70% alcohol.

The eggs were next carried through separately by the celloidin method with some modifications. They were removed from the 70% alcohol and placed into 95% then into 98% until fully dehydrated; one day for each was considered long enough. Then for a number of hours successively into alcohol-ether and ether, then transfered to thin celloidin, which was gradually allowed to thicken and at the end of two weeks imbedded, hardened in chloroform vapors and kept in 80% alcohol until ready for sectioning.

Another method of fixation was used with good results. The eggs were tipped as before and immersed in Bouin's fixative contained in a vial; but instead of fixing the egg whole the yolk contents were squeezed out. This was easily done by bringing pressure upon the capsule with a pair of forceps as the egg lay against the sides of the vial. It fixes very rapidly and no yolk breaks away. Moreover they infiltrate and section nicely, making the best sections of any in the lot.

However, the observer is not convinced that he is justified in using this technique for eggs in the early stages, as there is too much danger of displacing the cells, since they are more or less isolated. Whether it would serve a purpose for partly developed embyros, where the cells have become united, is also a question, because eggs of later stages were not fixed by this method.

The material was sectioned thirty micra thick with a sliding microtome. Each section as it was cut, was transfered to a slide and the sections arranged in series. They were then dehydrated with 98% alcohol and any excess alcohol removed from the slide. Now by putting a few drops of a solution, consisting of equal amounts of 98% alcohol and ether, on these sections and thus partly dissolving the celloidin, they were made to adhere firmly to the slide. If one application of alcohol-ether was not sufficient several were made, until the celloidin had lost its smooth surface and had assumed a rather rough appearance. When the alcohol-ether had evaporated almost to dryness the slide was with safety immersed into 95% alcohol and passed down the series for staining.

The sections were stained for 30-50 sec. in Delafield's haematoxylin, washed in tap water, passed up the series to 95% alcohol, counterstained with eosin, dehydrated with 98%, cleared in clearing oil ((three parts oil of thyme to one part oil of cloves); here one must proceed cautiously , for there is danger of loosening the sections from the slides. They were next passed through xylol and mounted in balsam.

The yolk material has a characteristic, perforated, polyhedral appearance and stains a pinkish red. The cells stain blue, the nuclei quite dark and they can be easily distinguished from the surrounding material. No differential chromatin stains were used as the material was prepared with the view of studying tissues. Few mitotic figures were fouhd and these were in the spireme stage.

OBSERVATION AND DISCUSSION:- The growth of the egg before it leaves the ovary will first be described. The ovary itself lies oh the dorsal side of the abdominal cavity and consists of a series of tubules in which the eggs are developed. Fig.1. shows one of these tubules in longitudinal section. The tube itself is composed of connective tissue surrounding the string of egg cells. At the proximal end of this tube the cells are small

and mitotic figures are greatly in evidence, in fact every cell shows some stage of mitosis. They rapidly increase in size, however, and before they advance very far from the proximal end of the tube they are arranged in a single row, each succeeding cell being somewhat larger than the preceeding. This increase in size is due to an increase in amount of cytoplasm, for the nuclei apparently remain unchanged. Before the cells are arranged in the manner described in the egg tube, mitosis of the egg nucleus ceases and as far as the egg cells have been followed out before deposition, the nuclei remain unchanged and the chromatin retains that characteristic reticulated appearance of a resting cell. A number of smaller cells are evident, lying near the periphery of the egg tube. These increase in number and when the egg cells are found arranged in single order these smaller cells are seen to crowd in between them. Soon they completely surround the egg cell and later form the capsule of the egg.

Such is the condition in fig. 2. where these cells in the form of a capsule completely surround the egg cell. They are uniform in size and may be classed as cells belonging to the low columner type.

As yet they exibit no evidence of function other than that of forming a covering for the egg dell. They increase in number for various mitotic figures can be seen in different parts of the capsule.

This condition soon changes. The cells of the capsule become elongated and assume another function. Fig. 3. shows such an egg. It contains in its cytoplasm an indefinite number of globules more numerous at the periphery than at the central axis of the egg. These are yolk globules and they invade the cytoplasm from the outside. The yolk is not laid down in the center as Wieman has found it in Crysomelid eggs. This, however, no doubt is due to the fact that in Crysomelid eggs there is a food stream coming from a specialized nurse cell while in Mermiria bivittata the cells making up the capsule act as nurse cells and secrete the yolk. I am led to this conclusion by the fact that the cells of the capsule have their nuclei crowded to the end and the cell content between the nucleus and the internal limiting wall of the cell is filled with a material staining identically with that of the yolk globules already laid down in the cytoplasm. In appearance these cells do not differ materially from somatic body cells of the human that have a secretory function such as the goblet cells of the alimentary tract. But whether these yolk secreting cells pour out their supply into the

cytoplasm like the goblet cells or whether it finds its way inward by a process of osmosis was not detirmined. No evidence was found showing a ruptured internal cellular wall, yet, the cytoplasm in the various cells reacted differently toward the stains; showing that the yolk content was unequal in cells of the same capsule. This indicates that the deposition of yolk is probably due to osmosis.

At the broad cephalic pole a peculiar condition exists. It is evidently the place where the nutritive products are primarily transmitted to the egg and where the increase in the size of the capsule takes place. The cells at this pole are very much elongated, spindle shaped in fact. (see fig.8.) They show no evidence of having a secretory function whatever, but the appearance of the cells in the immediate vacinity toward the distal pole seem to indicate that these cells are slowly differentiated into the capsular cells, for, there exist definite gradations from the long spindle shaped cells to those columnar yolk secreting cells of the capsule.

The growth of the egg and the deposition of yolk apparantly exercise no influence upon the germ nucleus whatever. The increase in the size of the egg varies

directlywith the amount of yolk laid down. One pole of the egg being attached, increased growth of the capsule will tend to force the other (distal) pole out. This being the case the egg "grows away" from the nucleus and the nucleus does not travel to the pole as was suggested by Wheeler,'92. I believe that I am justified in making these statements, for the relative distance and relationship of the germ nucleus to the capsule is practically the same in the various stages studied. Compare figs. 2, 3, and 4, which show germ nuclei at different stages in the growth of the egg yet their relation to the capsule are similar.

Figs. 4 and 5 were taken from the same egg, three sections intervening. Fig. 4 shows the arrangement of the cells at the cephalic pole around the micropyle, while Fig. 5 again shows the germ nucleus at the broad pole of the egg.

A gap must be left in the discussion at this point, for, although the material containing the maturation and fertalization stages has recently been collected, it has not yet been prepared for the microscope. Hence, a description of these stages will appear in a future paper.

Fig.6 shows the germ cell of an egg found not more than 12 hours after deposit. This nucleus, too, was found near the periphery of the egg at the broad pole. There is here no evidence of division, although the cytoplasm shows several things worthy of mention. The outline of the cell is somewhat irregular, containing several large vesicles. Imbedded in the cytoplasm and contained in some of these vesicles are found a number of yolk globules. This was the only cell found in the egg; therefore, it must be the germ cell. It shows no sign of division, yet it has assumed a function secondary in importance to that of reproduction, yet preceding it: that of a vitellophag.

This cell does not remain in this condition long, for, in an egg fixed the second day after deposition, seven cells were found, two of which are seen in fig.7. In this series of sections no cell was found occupying a central polar position, such as is shown in figs. 3 and 5, but the seven cells were destributed somewhat irregularly through the yolk at one pole. I conclude from this that they are the progeny of the germ cell and function as vitellophags. All cells held a position nearer the periphery of the yolk than a line

representing the central longitudinal axis of the egg. Neither of the cells had traveled farther than one third of the distance to the opposite pole. Again no satisfactory evidence of mitosis was obtained, although the cells were by no means uniform in appearance, two of them seemed to contain irregular darker staining nodules in their nuclei.

These vitellophags continue their course to the periphery of the yolk, (fig.9.) where they are seen lodged in small cavities , quite uniform in number with their cytoplasmic processes extending into the yolk, No union of the cytoplasm of one cell with that of another could be made out at this stage.

These vitellophags next form, with increase in number through tangential division, a definite union covering the entire surface of the yolk. This is the blastoderm. Fig.10 was taken from a tangential section of an egg showing the surface view of the blastoderm. These cells are large and polyhedral in appearance; their discoid shaped nuclei staining deeply, making a sharp contrast with the surrounding cytoplasm. Wheeler, in his work on Xiphidium, stated that the blastodermic cells divided by amitosis. No

case of this kind was observed in the blastoderm of Mermiria, but a few cells were noted showing mitosis. It is posible that both types of division occur, although the latter is the only type noted by the observer.

There is another point on which Wheeler was undecided: whether the intravitelline cells found in the yolk were inhibited in their progress to the periphery of the yolk or whether they were formed by centripital division after the completion of the blastoderm. Observations on this material lead to the conclusion that the latter case is correct, for both eggs, from which figs.9 and 10 were taken, showed no vitelline cells except those lying at the periphery of the yolk.

It is at this piont where Wheeler takes up the work on Xiphidium and it does not appear necessary to describe any of the stages that he has so carefully worked out.

SUMMARY:- The egg cells of Mermiria bivittata are developed by mitosis in an egg tubule, where they are found in irregular order. When arranged in single order, mitosis in those cells ceases.

The egg capsule is derived from cells, differentiated for the purpose, lining the egg tubule.

These capsular cells are further differentiated into yolk secreting cells with the growth of the egg.

The yolk invades the cytoplasm from the outside and is gradually crowded inward until the cytoplasm is completely filled.

During the growth of the egg, the egg nucleus maintains a relatively constant position with reference to the micropyle.

Maturation and fertilization stages were not found,

The germ cell after the deposition of the egg is found at the broad pole near the micropyle. It first acts as a vitellophag, then gives rise to progeny, which travel to the surface of the yolk exercising the same function. These unite at the surface of the yolk by tangential division and form the blastoderm from which the embryo is derived.

Cells of the blastoderm increase in number, at least in part, by mitosis.

Vitellophags seen in the yolk after the formation of the blastoderm are derived from the same by centriptial division.

EXPLANATION OF PLATE 1.

Fig.1. Longitudinal section of ovarian tubule showing early development of egg cells. m- Cells showing mitosis; cc- Connective tissue cells; ec- Egg cell in the growing stage.

Fig. 2. Crosssection of egg cell before the deposition of yolk. c- Cells of capsule- derived from cc. in fig. 1. m- mitosis.

Fig .3. Egg cell showing deposition of yolk. n- nucleus; mi- micropylar pole ; cy- cytoplasm; y- yolk globules deposited in the cytoplasm.

Fig. 4. Cephalic or broad pole of egg. c.m.- cells surrounding micropyle.

Fig. 5. Shows the egg nucleus at the periphery of the egg; very little cytoplasm but abundance of yolk material present. n- egg nucleus;y- yolk; cy- cytoplasm; c-cells of capsule.

Fig. 6. Highpower drawing of the germ cell found at the broad pole of the egg showing yolk inclusions at (y).

PLATE - 1 -

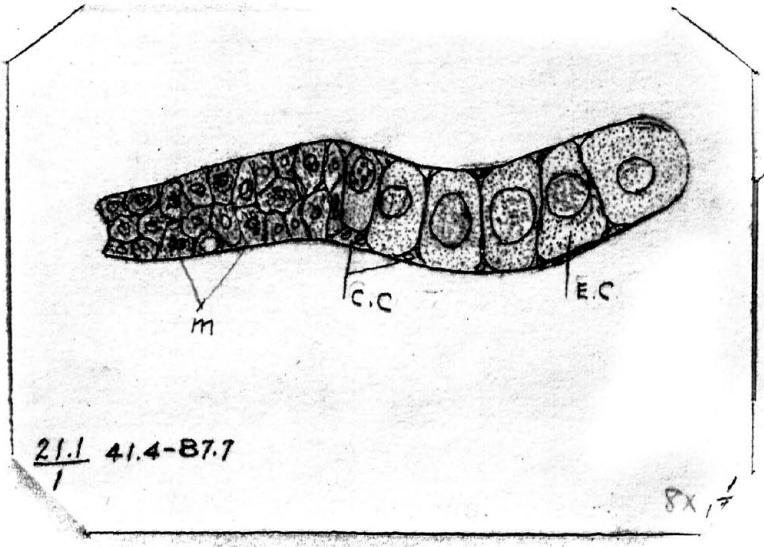

Fig. 1.

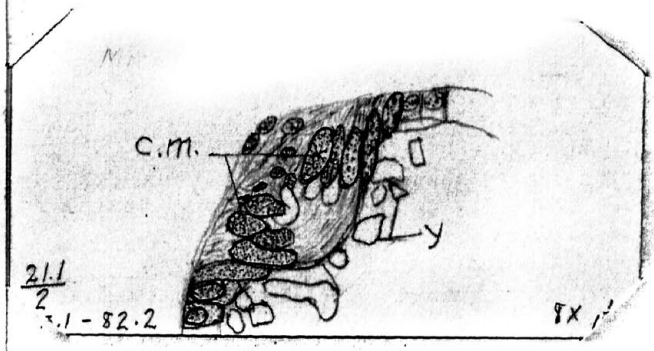

Fig. - 4.

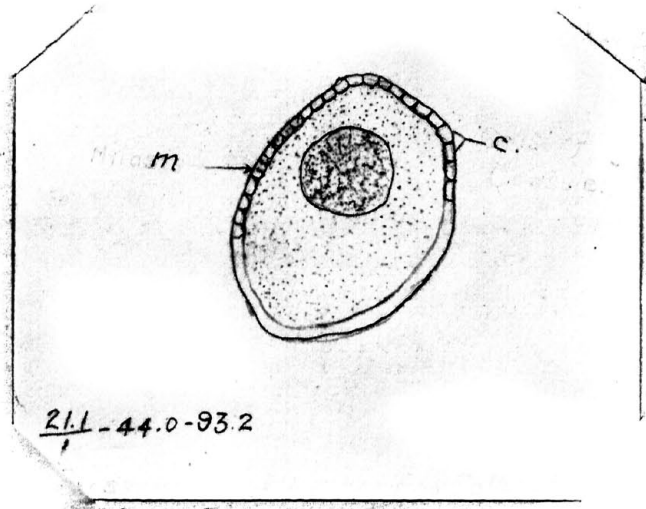

Fig. 2.

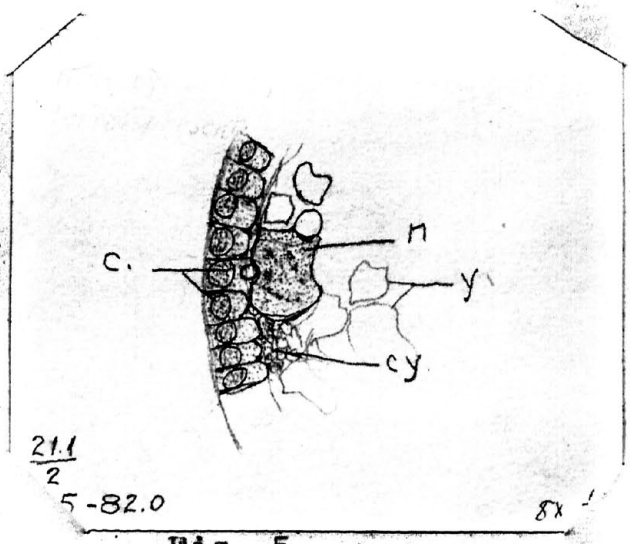

Fig. 5.

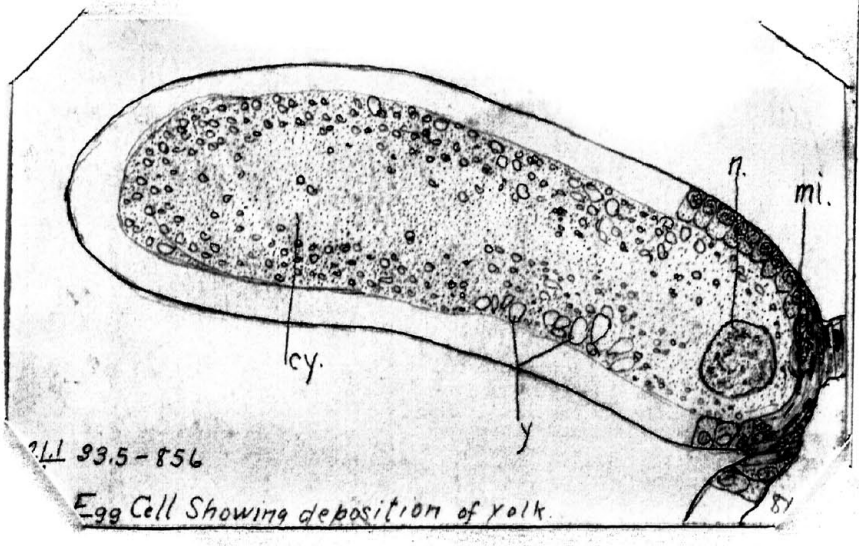

Fig 3.

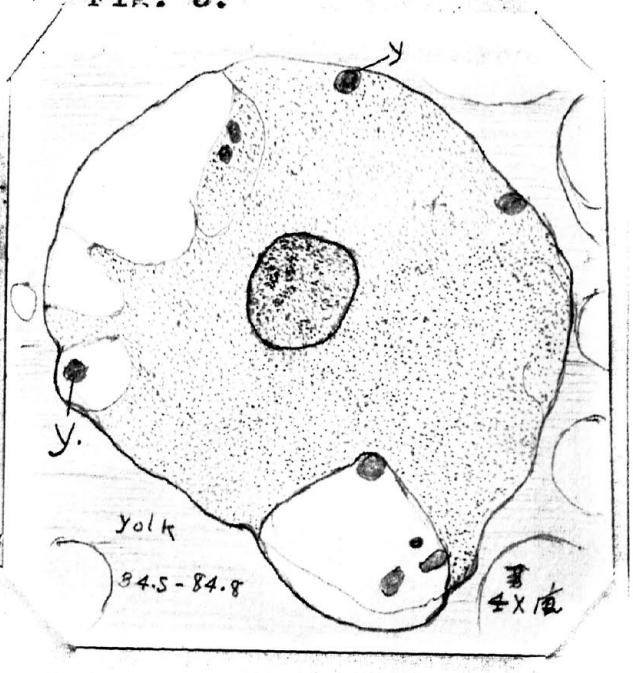

Fig 6.

EXPLANATION OF PLATE II.

Fig. 7. Intravitelline cells, derived from the germ nucleus, on their way to the periphery of the yolk. vi. Intravitelline cells; y. periphery of yolk; c. internal limiting wall of capsule.

Fig. 8. Drawing from the broad pole of the capsule to show the differentiation of the capsular cells. s.c. spindle shaped cells at extreme end of capsule; i.s. intermediate stage; y.c. fully formed yolk secreting cells of capsule.

Fig. 9. Intravitelline cells at periphery of yolk acting as vitellophags and forming blastoderm. m.c. Inner margin of capsule; vit. Intravitelline cell; yo. yolk.

Fig. 10. Surface view of fully formed blastoderm.

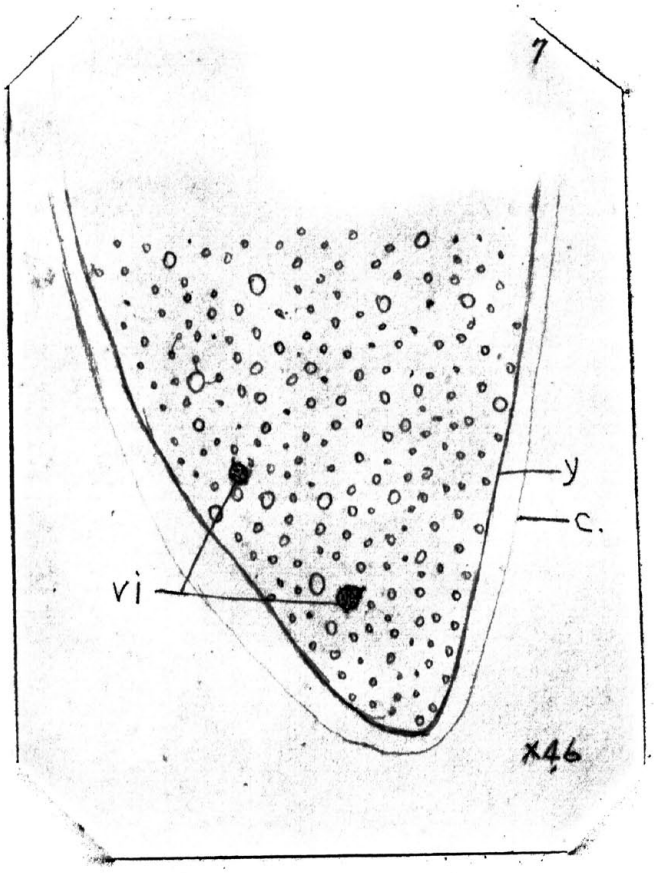

Fig. 7.

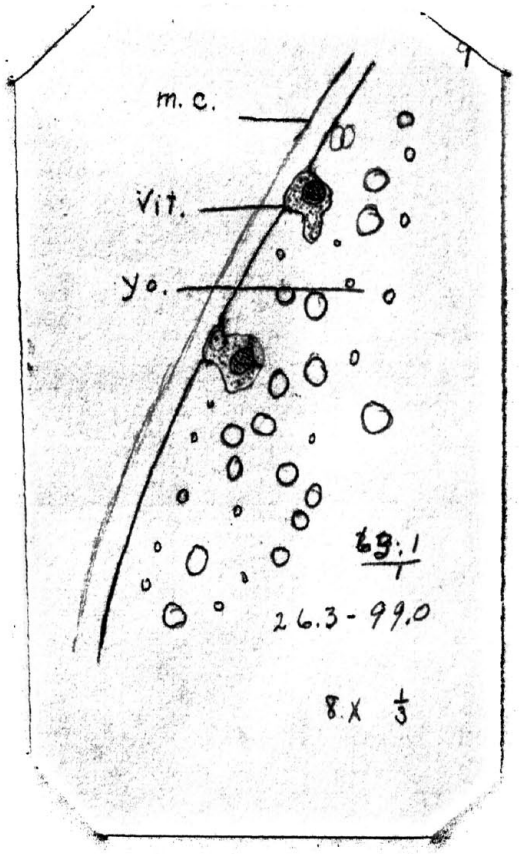

Fig. 9.

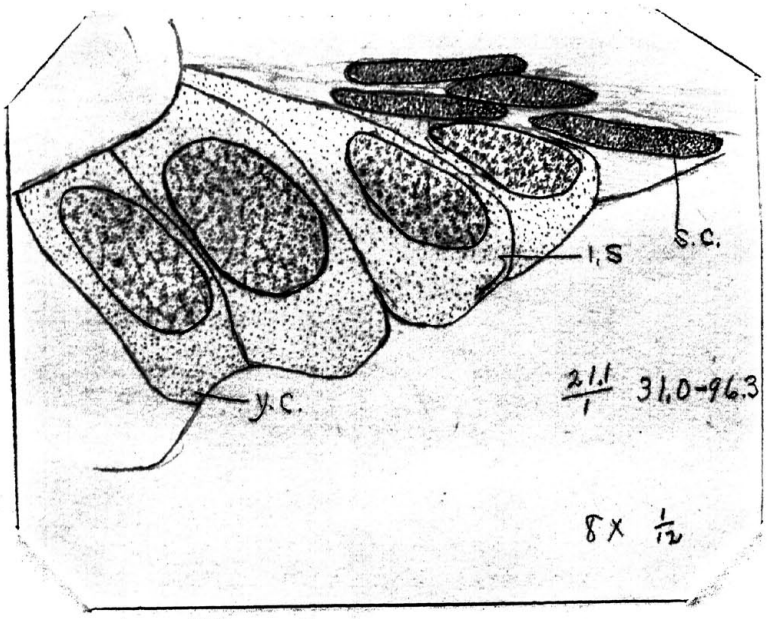

Fig. 8.

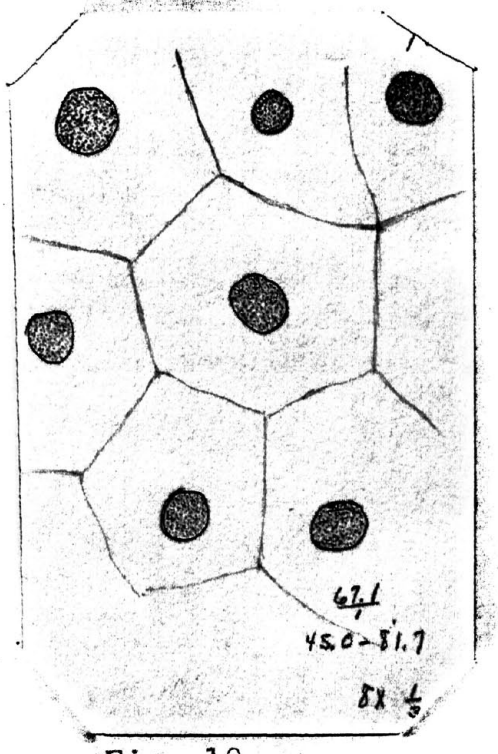

Fig. 10.